U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER
KORSGREN=1

TRANMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
09/890936

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY CLAIMED
PCT/SE00/00223	04 February 2000	05 February 1999

TITLE OF INVENTION
NOVEL USE WITHIN TRANSPLANTATION SURGERY

APPLICANT(S) FOR DO/EO/US
Olle KORSGREN et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] The US has been elected in a Demand by the expiration of 19 months from the priority date (PCT Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not transmitted by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. [X] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C 371(c)(3))
   a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An Assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter
16. [X] Other items or information:
    [X] Courtesy copy of the International Application as filed.
    [X] Courtesy copy of the first page of the International Publication (WO 00/45837).
    [X] Courtesy copy of the International Preliminary Examination Report. There were no annexes.
    [X] Formal drawings, 3 sheets, Figures 1-3.
    [X] Courtesy Copy of the International Search Report.
    [X] Application Data Sheet
    [X] The application is (or will be) assigned to: CorlineSystems AB, whose address is Box 956, S-751 09 Uppsala, Sweden.

U.S. APPLICATION NO (If known, see 37 CFR 1.5)	International Application No	Attorney's Docket No
09/890936	PCT/SE00/00223	KORSGREN=1

17. [xx] The following fees are submitted.

	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR 1.492 (a)(1) –(5): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. **$1000.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO. **$860.00**	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..... .....**$710.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).....**$690.00**	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4).....**$100.00**	

ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 1,000.00
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [X] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$ 130.00
Claims as Originally Presented	Number Filed	Number Extra	Rate	
Total Claims	17 - 20		X $18.00	$
Independent Claims	3 - 3		X $80.00	$
Multiple Dependent Claims (if applicable)			+$270.00	$ 270.00
TOTAL OF ABOVE CALCULATIONS =				$ 1,400.00
Claims After Post Filing Prel. Amend	Number Filed	Number Extra	Rate	
Total Claims	- 20		X $18.00	$
Independent Claims	- 3		X $78.00	$
TOTAL OF ABOVE CALCULATIONS =				$ 1,400.00
Reduction of ½ for filing by small entity, if applicable. Applicant claims small entity status. See 37 CFR 1.27.				$ 700.00
SUBTOTAL =				$ 700.00
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$
TOTAL NATIONAL FEE =				$ 700.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				$
TOTAL FEES ENCLOSED =				$ 700.00
			Amount to be: refunded	$
			charged	$

a. [ ] A check in the amount of $_____ to cover the above fees is enclosed

b. [X] Credit Card Payment Form (PTO-2038), authorizing payment in the amount of $ 700.00, is attached.

c [ ] Please charge my Deposit Account No. **02-4035** in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. [**XX**] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-4035**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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624 NINTH STREET, N.W., SUITE 300
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Date of this submission, **Monday, August 6, 2001**

SIGNATURE

Sheridan Neimark
NAME

20,520
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: KORSGREN=1

In re Application of: Olle KORSGREN et al.	)	Art Unit:
	)	Examiner:
I.A.. No.: PCT/SE00/00223	)	Washington, D.C.
Filed: 4 February 2000	)	August 6, 2001
For: NOVEL USE WITHIN…	)	

PRELIMINARY AMENDMENT

Honorable Commissioner for Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE SPECIFICATION

After the title please insert the following paragraph:

REFERENCE TO RELATED APPLICATIONS

--The present application is the national stage under 35 U.S.C. 371 of international application PCT/SE00/00223, filed February 4, 2000 which designated the United States, and which international application was published under PCT Article 21(2) in the English language.--

REMARKS

The above amendment to the specification is being made to insert reference to the PCT application of which the present case is a U.S. national stage.

Favorable consideration is earnestly solicited.

Respectfully submitted,
BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant

By: _____
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Title: Novel use within transplantation surgery

Field of the invention

The present invention is within the field of transplantation surgery. More closely, the present invention relates to use of a clotting preventing agent in the production of a drug for administration in association with transplantation of cells and tissue, such as insulin producing cells to patients with insulin dependent diabetes mellitus, IDDM.

Background of the invention

The only option to achieve permanent normoglycemia in diabetic patients is a renewal of the β-cells, either by transplantation of segmental/whole pancreas or isolated islets of Langerhans. Transplantation of isolated islets is considerably less successful compared to whole pancreas transplantation. The immunological barrier, the underlying autoimmune disease and the immunosuppressive drugs used, are the same in both types of transplantation. Thus, there is no obvious immunological explanation as to why transplantation of whole pancreas is more successful than islet transplantation.

If, however, the problems related to the unsuccessful outcome of transplantation of islets were identified and a technical and practical solution was developed, obvious benefits for the patients would be created implying interesting commercial opportunities.

The prior art in this field is largely confined to measures aiming at reducing immunological reactions. WO 9711607 describes transplantation of microencapsulated insulin producing cells as a means of protecting the cells from immunological reactions and/or combined with treating the recipient with a substance that would inhibit an immune-system costimulation. WO 9105855 describes transplantation of islets of animal origin and that the

animal tissue should be modified to contain homologous complement restriction factors. DE 19623 440 A1 describes methods for encapsulation of islets and points out that the artificial encapsulation material may induce platelet activation, coagulation and complement activation, and therefore the encapsulation material should be modified to allow release of inhibiting substances as e.g., heparin, hirudin or Marcumar. US 5 635 178 is not related to transplantation of islets but describes monoclonal antibodies having inhibitory activity towards the terminal complex of complement and that such antibodies can be used to reduce activation of platelets and endothelial cells.

It is evident for those skilled in the art that measures aiming at inhibiting immunological reactions in connection with transplantation of islets regardless of being allogenic or xenogenic have not lead to a satisfactory result in respect of clinical outcome.

Summary of the invention

The present inventors have performed experiments implying adding human, adult porcine or fetal porcine islets to human whole blood and have been struck by the vigorous coagulation occurring when these islets were injected into human ABO-compatible blood. As judged by microscopical examinations it is evident that the islets are rapidly coated by a layer of platelets which soon develops into an organised thrombus. This biological event has previously not been considered and is now suggested to be a major explanation as to why the outcome of autologous islet transplantation has been comparatively unsuccessful. The present invention is related to measures to reduce this incompatibility reaction that can either be directed towards inhibiting activation of platelets, mono- or polymorhonuclear cells or the enzyme cascade of coagulation. Regardless of the initiating event, any of these reactions will lead to generation of thrombin, which eventually converts fibrinogen to fibrin. The generation of thrombin can easily be monitored by measuring the thrombin- antithrombin complex (TAT complex).

Hence, the present invention is concerned with therapeutic measures to inhibit TAT complex formation upon exposure of allogenic or xenogenic islets to whole blood.

Therefore, the present invention relates to a use of a clotting preventing agent in the production of a drug for administration in connection with transplantation of cells and tissue, such as insulin producing cells in the form of isolated islets to patients with insulin dependent diabetes mellitus, IDDM.

Preferably, the clotting preventing agent is an anticoagulant, such as heparin or fractions or derivatives thereof. Alternatively, hirudin, oxalate, citrate etc. can be used.

In one embodiment of the invention, the islet cells are coated with heparin or fractions or derivatives thereof by preincubation of islets in a solution containing heparin or fractions or derivatives thereof. Using a conjugate of heparin to coat the islets, it was demonstrated that the modified islets had acquired an increased capacity to adsorb antithrombin and loop experiments (described below) demonstrated that it is possible to reduce clotting by using such modified islets.

In an alternative embodiment of the invention, the preventing agent is an inhibitor of platelet activation, such as a RGD (standard one letter code for amino acids) containing peptide or a monoclonal antibody which inhibits the interaction of platelet integrins with their specific ligands. This antibody is for example a monoclonal antibody or a peptide directed against the Fc receptor on platelets.

A combination of anticoagulant and inhibitor of platelet activation can be used as clotting preventing agent according to the invention or any other suitable combination of preventing agents. Optionally, the preventing agent(s) is/are supplemented by an inhibitor of complement.

Furthermore, the invention relates to a method for increasing survival of islet cells in connection with transplantation of insulin producing cells to patients with insulin dependent diabetes mellitus, IDDM, comprising prevention of clotting, monitored as reduced generation of thrombin-antitrombin complex.

Detailed description of the invention

The invention will be described more closely below in association with the accompanying drawings, in which

Fig. 1 is a graph showing percent aggregation of platelets following addition of islets to platelet rich plasma, PRP, as a function of time;

Fig. 2 shows a similar graph as in Fig. 1 but here a RGDS (standard one letter code for amino acids) tetrapeptide was added to PRP before islets were added; and

Fig. 3 shows a similar graph as in Figs. 1 and 2 but here a monoclonal antibody against the Fc receptor on platelets was added to PRP before islets were added.

All the in vitro experiments for studies of islets contacting whole blood were performed in a tubing loop model. The experimental model is a modification of a model for testing biomaterials that has previously been described (J. Clin. Immunol. 16, 223-230 (1996)). Tubings made of polyvinylchloride (PVC, i.d. = 6.3 mm, length = 300 mm) were modified with immobilized heparin according to a method developed by Corline Systems AB (Uppsala, Sweden) as disclosed in international patent application no WO93/05793. Briefly, the polymer surface is modified with a high molecular weight amine compound to add primary amine groups to the surface. A soluble conjugate prepared by covalent binding of approximately 60 mol of heparin per mol of a straight-chained polyallylamine is irreversibly bonded onto the amine surface of the tubings. This procedure results in a total surface

concentration of heparin of approximately 0.5 μg/cm^2. By using such heparin modified tubings it is possible to incubate the tubings with non-anticoagulated fresh human blood in a rocking device at 37°C for one hour with only moderate activation of blood (c.f. control column in Table 1 A and 1 B below). Unmodified tubings will invariably induce complete clotting at these experimental conditions. Addition of human islets or porcine adult or fetal islets lead to some remarkable observations. Complete clotting invariably occurred with a total loss of platelets, a sharp increase in the formation of TAT and a very significant increase in the markers of the early contact phase (FXIIa and FXIa) of coagulation (C.f. Table 1 A and 1 B). Histological examination revealed a dense layer of activated platelets immediately adjacent to the capsule of the islets.

The findings in vitro described above were confirmed in vivo by evaluation of porcine islets after intraportal transplantation in pigs. The porcine livers, removed 60 min. after islet transplantation, had a congested appearance with patchy dark discoloration's on the surface. In the portal veins blood clots were found, with a patchy adherence to the endothelium, and branching into the portal tree, partially occluding the vessels. The histological examination revealed islets entrapped in blood clots, with a disrupted islet morphology. Occasionally a fibrin tail could be observed extending away from the islet.

With reference to Table 1 B, it appears that the effect of adding an inhibitor of complement leads to reduced activation of complement, as expected, but there is no measurable effect on the clotting of blood or activation of platelets. If, however, soluble heparin was added to the experimental system there was a remarkable improvement in preservation of the number of platelets and reduced generation of TAT.

In another set of experiments the effects of inhibiting the interaction between platelet integrins and their specific ligands were investigated. With reference to Fig. 1-3, it appears that platelet aggregation is induced upon contact with

islets and that such aggregation can be prevented by blocking platelet integrins or Fc-receptors.

Porcine islets were surface modified by incubation in a buffered solution containing a high molecular weight conjugate of heparin (Corline Heparin Conjugate), as disclosed in WO 93/05793, and then rinsed by changing buffer several times. It was demonstrated that the modified islets had acquired an increased capacity to adsorb antithrombin and loop experiments showed that heparin modified islets resulted in reduced clotting compared to unmodified islets.

It is easily understood by those skilled in the art that there is a broad arsenal of agents that can be used to accomplish reduced clotting, and hence, the following non-limiting Examples are only used to demonstrate the principle behind the present invention.

Example 1: Effect of soluble heparin

Sixty ml of non-anticoagulated blood was collected from healthy blood donors using heparin-coated equipment. U-shaped tubings with a total volume of nine ml were filled with eight ml of blood immediately followed by addition of isolated human islets or porcine adult or porcine fetal islets (500 IEQ). The tubings were then closed into loops using connectors of titanium furnished with immobilised heparin. The tubing loops were placed vertically in a rocking device and the complete apparatus was placed in an incubator at 37°C for up to sixty minutes. At the end of the rocking period blood was collected in EDTA and the number of cells were counted in a automatic cell counter. The blood samples were then centrifuged at 4°C (3290xg, 20 min) and EDTA plasma was collected and immediately put at –70°C. Islets retrieved after blood perifusion were prepared for immunohistochemistry. The results are summarized in Table 1A and 1B below.

Table I A shows results of blood cell counts and coagulation and complement parameters before and after 60 min. of human islet perifusion with ABO-compatible fresh human blood or blood supplemented with heparin.

Table 1A

Table 1A: Blood cell counts and coagulation and complement parameters before and after 60 min. of human islet perifusion with ABO-compatible fresh human blood or blood supplemented with heparin.

	BEFORE	CONTROL	HUMAN ISLETS	
			WITHOUT ADDITIVES	HEPARIN
Platelets ($x10^9$)	233 ± 13.8	161.1± 9.3	5 ± 0.3***	114 ± 17*
Neutro. ($x10^9$)	3.23 ± 0.33	3.03 ± 0.32	0.83 ± 0.18***	2.56 ± 0.43
Mono. ($x10^9$)	0.36 ± 0.03	0.36 ± 0.04	0.03 ± 0.01***	0.28 ± 0.06
Lymph. ($x10^9$)	1.91 ± 0.12	1.77 ± 0.12	1.29 ± 0.12**	1.60 ± 0.20
C3a (ng/mL)	84 ± 4.7	507 ± 115	1259 ± 125.1***	565 ± 143.6
C5b-9 (AU/mL)	15.6 ± 2.9	95 ± 30	213 ± 43.4*	147 ± 39.6
FXIIa-AT (umol/L)	0.09 ± 0.01	0.36 ± 0.15	12.9 ± 0.9***	5.4 ± 1.7**
FXIa-AT (umol/L)	0.06 ± 0.01	0.12 ± 0.03	4.74 ± 0.48***	0.34 ± 0.12*
TAT (ug/mL)	12.5 ± 5.2	316 ± 100	20537 ± 1973***	4467 ± 2285

Control loops contained blood and culture medium (RPMI), but no islets. All values are stated as the Mean ± SE(M). TAT, Thrombin-antithrombin. The degree of significance is reported with respect to the controls.
(*p<0.05; **p<0.01; ***p<0.001; n.a. = not analysed).

Table I B shows results of blood cell counts and coagulation and complement parameters before and after 60 min. of adult and fetal porcine islet perifusion with fresh human blood or blood supplemented with the complement inhibitor C1 inactivator (C1-INA) or heparin.

Table 1B: Blood cell counts and coagulation and complement parameters before and after 60 min. of adult and fetal porcine islet perifusion with fresh human blood or blood supplemented with C1-INA or heparin.

	BEFORE	CONTROL	ADULT ISLETS			FETAL ISLETS
			WITHOUT ADDITIVE	C1-INA	HEPARIN	WITHOUT ADDITIVES
Platelets ($x10^9$)	237± 8.0	171 ± 9.0	4 ± 0.1***	4 ± 0	145 ± 13.0	4 ± 0***
Neutrophils ($x10^9$)	2.75 ± 0.21	2.52 ± 0.21	0.57 ± 0.07***	0.41 ± 0.13	3.00 ± 0.19	1.44 ± 0.17**
Monocytes ($x10^9$)	0.38 ± 0.02	0.37 ± 0.02	0.04 ± 0.01***	0.15 ± 0.01	0.35 ± 0.05	0.07 ± 0.01***
Lymphcytes ($x10^9$)	2.30 ± 0.14	2.13 ± 0.11	1.74 ± 0.10*	1.23 ± 0.38	1.88 ± 0.11	1.68 ± 0.25
C3a (ng/mL)	80.1± 7.3	545 ± 68	1435 ± 173***	1094 ± 78	486 ± 139	1601 ± 215***
C5b-9 (AU/mL)	15.8 ± 1.8	72 ± 10	283 ± 34***	183 ± 29	82 ± 22	302 ± 46***
FXIIa-AT (mmol/L)	0.18 ± 0.03	0.13 ± 0.00	8.96 ± 1.38***	19.65 ± 0.45	3.56 ± 1.60**	n.a.
FXIa-AT (mmol/L)	0.04 ± 0.00	0.03 ± 0.00	4.14 ± 0.48***	2.95 ± 0.15	0.53 ± 0.26	n.a.
TAT (ug/mL)	5.6 ± 1.1	139 ± 35	23886 ± 3494***	30250 ± 3450	505 ± 162***	34420 ± 4875

Control loops contained blood and culture medium (RPMI), but no islets. All values are stated as the Mean SE(M). TAT, Thrombin-antithrombin. The degree of significance is reported with respect to the controls.
(*p<0.05; **p<0.01; ***p<0.001; n.a..= not analysed).

C1 inactivator reduced complement activation but had no detectable effect on the coagulation parameters. Soluble heparin, however, prevented clotting and there was a remarkable improvement with respect to platelet count and generation of TAT. Notwithstanding the results obtained by the use of C1-INA, it is obvious that it should be beneficial to combine an anticoagulant with an inhibitor of complement.

Example 2: Effect of platelet inhibitor

Platelets in platelet rich plasma (PRP) and gel filtered platelets were tested in an aggregometer. Islets were added to PRP and thereafter analysed in the aggregometer. It was shown that the islets induced aggregation of the platelets (Fig. 1) and that platelets number in the sample were reduced from 375 x 10^9 to 236 x 10^9. If purified platelets without plasma proteins were used in combination with islets no aggregation and reduction in the platelet count were observed. In attempts to identify the mechanism behind the induced aggregation, an RGDS tetrapeptide to inhibit integrin binding and a monoclonal antibody against Fc recpetors on platelets were used. Addition of the RGDS peptide totally abolished the aggregation and the consumption of platelets when islets were added to PRP (Fig. 2). A similar finding was obtained if the anti-Fc receptor antibody was added (Fig. 3).
Conclusion: The experiments show that islets bind to platelets when added to PRP. This binding induce activation and aggregation of the platelets.

Example 3: Effect of surface modification of islets using a heparin conjugate

Using Corline Heparin Conjugate (c.f. WO 93/05793) containing approximately sixty mol of heparin covalently bound to one mol of straight-chained carrier, adult porcine islets were modified by irreversible adsorption of the heparin conjugate onto the surface of the islets. This was

accomplished by incubating the islets for 30 minutes at 37°C in a buffered saline solution containing heparin conjugate.

The presence of heparin at the surfaces of the islets was demonstrated by an ELISA assay for islet surface associated antithrombin (AT). Unmodified and heparin modified islets were incubated in human plasma for thirty minutes and then rinsed several times by changing buffer. The islet were then incubated with anti-AT that had been labelled with biotin. Using HRP-labelled streptavidin the uptake of anti-AT could be semiquantitatively estimated. The uptake of anti-AT on the heparin modified islets was three times higher than that on the unmodified islets showing that biologically active heparin was present on the surface of the islets. Testing of heparin modified islets in the tubing loop model resulted in less clotting compared to unmodified islets.

The present invention is expected to significantly improve the situation for IDDM patients. By administering an anticoagulant and/or inhibitor of platelet activation, optionally in combination with surface modification of islets, and optionally together with a complement inhibitor, in association with transplantation of insulin producing cells it is expected that the need of providing these patients with injections of insulin will be substantially decreased or even eliminated.

CLAIMS

1. Use of a clotting preventing agent in the production of a drug for administration in connection with transplantation of insulin producing cells in the form of isolated islets to patients with insulin dependent diabetes mellitus, IDDM.

2. Use according to claim 1, wherein the preventing agent is an anticoagulant.

3. Use according to claim 2, wherein the anticoagulant is heparin or fractions or derivatives thereof.

4. Use according to claim 3, wherein islet cells are coated with heparin or fractions or derivatives thereof by preincubation of islets in a solution containing heparin or fractions or derivatives thereof.

5. Use according to claim 1, wherein the preventing agent is an inhibitor of platelet activation.

6. Use according to claim 5, wherein the preventing agent is a RGD containing peptide or a monoclonal antibody which inhibits the interaction of platelet integrins with their specific ligands

7. Use according to claim 5, wherein the preventing agent is a monoclonal antibody or a peptide directed against the Fc receptor on platelets.

8. Use according to any of the above claims, wherein more than one preventing agent is used.

9. Use according to any of the above claims, wherein the preventing agent(s) is/are supplemented by an inhibitor of complement.

10. Isolated cells comprising islets of Langerhans, characterized by being coated with a heparin conjugate on the islet surface.

11. A method for increasing survival of islet cells in connection with transplantation of insulin producing cells to patients with insulin dependent diabetes mellitus, IDDM, comprising prevention of clotting, monitored as reduced generation of thrombin-antitrombin complex.

Fig. 1

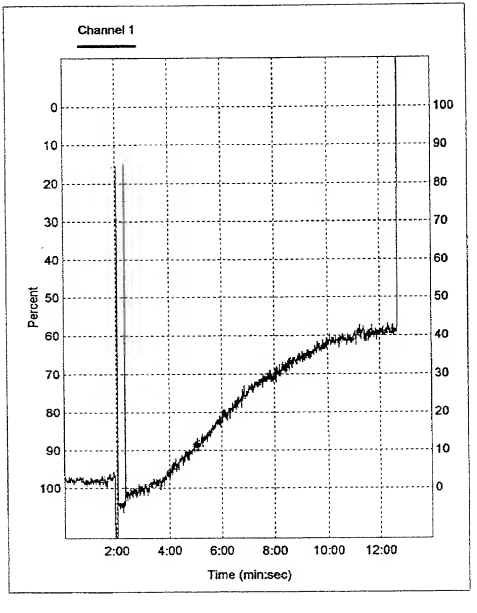
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Fig. 2

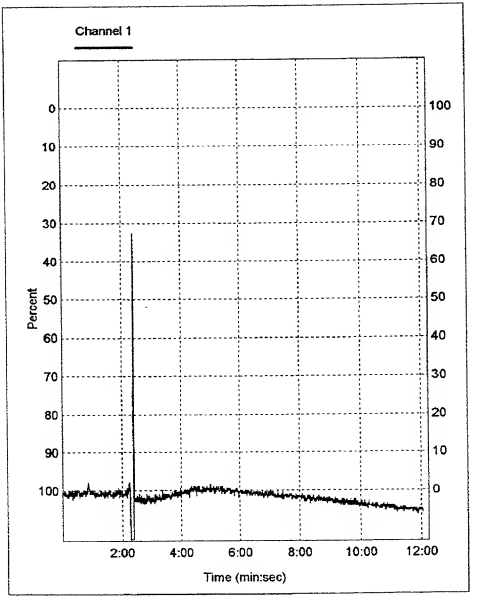
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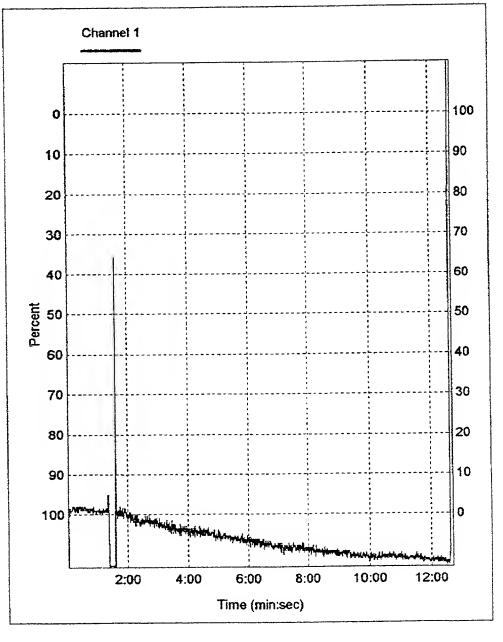
Fig. 3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Page 1 of 2 Pages [X] Original [ ] Substitute [ ] Supplemental Atty. Docket: KORSGREN=1

Combined Declaration for Patent Application and Power of Attorney

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

NOVEL USE WITHIN TRANSPLANTATION SURGERY

the specification of which (check one)

[ ] is attached hereto;
[ ] was filed in the United States under 35 U.S.C. §111 on , as U.S. Appln. No. _____*; or
[X] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/SE00/00233; filed 04 February 2000, entry requested on August 6, 2001*; national stage application received U.S. Appln. No. 09/890,936 *; §371/§102(e) date _____* (* if known)

and was amended on August 6, 2001 (if applicable).
(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119 (a)-(d) and 365 (b) of any prior foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or under §365(a) of any PCT application which designated at least one country other than the U.S., listed below:

Application No.	Country	Filing Date (MM/DD/YYYY)
9900398-0	Sweden	February 5, 1999

If I claimed foreign priority above, I hereby identify below any foreign application for patent (including an international (PCT) application designating a country other than the United States) or for an inventor's or plant breeder's certificate, having a filing date before that of the earliest application from which foreign priority is claimed (if left blank, then there are none):

Non-Priority Application No.	Country	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional applications listed below:

Application No.	Filing Date (MM/DD/YYYY)

I hereby claim the benefit under 35 U.S.C. §120 of any prior U.S. non-provisional application(s) or under §365(c) of any prior PCT international application(s) designating the U.S., listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT international application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information which is material to patentability as defined in 37 C.F.R. §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application No.	Filing Date (MM/DD/YYYY)	Status (patented, pending, abandoned)

As a named inventor, I hereby appoint the following registered practitioners to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

All of the practitioners associated with Customer Number 001444

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Page 2 of 2 Pages Atty. Docket: KORSGREN-1

Title: NOVEL USE WITHIN TRANSPLANTATION SURGERY

U.S. Application filed [ ], Serial No. 09/890,936

PCT Application filed February 4, 2000, Serial No. PCT/SE00/00223

The undersigned hereby authorizes the U.S. Attorneys or Agents appointed herein to accept and follow instructions from BRANN as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorneys or Agents and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents appointed herein will be so notified by the undersigned.

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.